Bacteriological Assessment Of The *Salmonella* Status Of Market-aged Pigs

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Abstract

The subclinical *Salmonella enterica* infection in pigs is associated with public health problems and can constitute a hindrance towards national and international trade. Many countries are starting the implementation of *Salmonella* national surveillance programs in pork. In spite of the increase of available data on the subject, no consensus seems to exist when assessing *Salmonella* status of finishing pigs.

This study aimed to find an alternative method to assess the status of finishing pigs. Following a first screening at the slaughterhouse, 4 farms were selected, 2 giving maximum positive results and 2 giving maximum negative results. From these 4 selected farms, the *Salmonella* status of 23 batches of finishing pigs was obtained with three sampling methods : fecal, environmental and serological.

Introduction

Salmonelle enterica infection in pigs is asymptomatic and does not concern animal pathology. Nonetheless studies in this infection are of increasing meaning with the appearance of outbreaks from pork products (1). In a way to control the quality of the products, we had to find the solution to assess the *Salmonella* status of marked-aged pigs. It appears that *Salmonella* found in slaughterhouses originated from pigs and not from resident strains (2). Thus, it is necessary to define the status of pigs at the herd. This assessment is based on bacteriological or serological evaluation of the animals. Although increased data are available on this subject, it seems to be difficult to assess the status of the pigs (3). As it is difficult to evaluate objectively the risk associated with the batch, we propose to use a bacteriological sampling method. This evaluation of the excretion excludes the individual variations (intermittent excretion) of fecal sampling and the delayed response of serology (with the risk of excluding pigs which do not excrete *Salmonella* anymore, or more problematic, not exclude pigs contaminate later). This method is a swabbing of the environment and we suggest it reflects the distribution of the pigs contamination.

Materials and Methods

Four farms, 2 giving maximum positive results and 2 giving maximum negative results, were selected from a preliminary study conducted at a slaughterhouse among 31 unrelated herds. Each month, for each selected farm, a batch of marked-aged pigs leaving the farm in the week was sampled to assess the *Salmonella* status of the batch.

Between 10 and 17 pigs (about 10 percent of the batch), evenly distributed in the different pen of a finishing room, were identified. Feces (25g) were obtained by individual rectal sampling.

The environmental samplings consisted in swabbing the walls of a pen at pig shoulder-height all around the pen. The mangers and eventually the soup mixer were swabbed to constitute a total of seven environmental samples for a finishing room. The swabs were humidified with 50 ml of phosphate buffered peptone water.

At the slaughterhouse, blood samples were obtained from the identified pigs giving the fecal sampling.

Bacteriological samples were pre-enriched in phosphate buffered peptone water (AES Laboratoire, Combourg, France) in 1:10 sample/broth ratio at 37°C for 18-20h. One hundred µl of this pre-enrichment broth were used to inoculate the middle of a Modified Semi-solid Rappaport Vassiliadis (MSRV) (Merck, Nogent sur Marne, France) agar plate and incubated at 41.5°C for 24h. From a migration zone superior to 20 mm, cultures were streaked onto Rambach agar plate (Humeau, La Chapelle sur Erdre, France). Rambach agar plate were incubated at 37°C for 24h. *Salmonella* typical colored colonies were confirmed by biochemical assay on Kligler Hajna medium (AES Laboratoire, Combourg, France) and then, serotyped by slide agglutination tests using *Salmonella* polyvalent O and H antisera (Diagnostic Pasteur, Paris, France).

The serological method was a complete indirect ELISA test based on mixed lipopolysaccharides of *Salmonella enterica* from the main serogroups isolated in finishing pigs in a previous study. Control sera were provided by the Danish Veterinary Laboratory. Sera were diluted 1:400, the secondary antibodies were peroxidase-conjugated rabbit anti-pig IgG antibodies. The reaction obtained with *o*-phenylenediamine as a substrate, was stopped with 0.5M

H2SO4, then coloration was read using a Dynatech MR5000 spectrophotometer (test filter 490 nm- reference filter 630 nm). Optical density was corrected with the positive and negative controls, the positive threshold value being 0.4.

Results

Farms A and B showed a high level of contamination as described by the three methods for the total results. Farms C and D showed a lesser contamination according to the three methods.

For farm A, the level of contamination was equally described by serology and the environmental sampling. The percentage of excreting pigs was about 30%. For farm B, serology and fecal sampling showed a level of positive results around 50%, the environmental sampling being a bit higher with 67% of positive samples.

For farm C and D, environmental and fecal sampling was below 15%, the serological sampling being a bit higher with 30% of positive samples.

Farm A showed a high level of contamination. Whereas the percentage obtained by serology reflected this positivity, the batch to batch heterogeneity of the excretion was better shown by the bacteriological sampling with an agreement at least in the trends. For example, the batch sampled in September was low for fecal and environmental bacteriology. And although the batch in July was not so high in fecal analysis, all the environmental swabbing were positive.

Farm B showed the most complex profile. Serological and bacteriological gave alternatively high and low values. For example the batch in June showed few bacteriological positive pigs, but the environment was contaminated and the percentage of seropositive pigs was high. On the other hand, in November, although the percentage of excreting pigs was high and well described by environmental swabbing, very few pigs had seroconverted.

For farm C, an episode of contamination between July and September appeared clearly. It was equally defined by the three methods. Interestingly, no time lag could be noted for the seroconversion. This contamination was better detected by environmental sampling than by the fecal sampling. The percentage of positive results was higher for serology. In November, the level defined by the three methods was low again.

Farm D was not or weakly contaminated during the six months of the study. The results obtained for the four first batches studied were equivalent using the three sampling methods. For the fifth batch, only one environmental sampling was positive but more than 70% of the pigs were seropositive.

Discussion

The environmental sampling procedure we used appears at least as discriminating as the individual fecal sampling. Moreover it assesses the contamination level without individual variations, requires less animal manipulation, and is easier to perform in the laboratory. The punctual inadequation of the serological and bacteriological assessment of the *Salmonella* status has previously been described (4). This could be explained by the fact that excretion and seropositivity are not necessarily correlated. Thus we could consider a serological assessment of the status of a batch more sensitive (with the exception of batches that had not yet seroconverted in the case of late contamination). And the specificity of the bacteriological assessment of the contamination validates the serological results.

An environmental assessment of the batch status is a reliable tool to identify the batch expected as a source of infection at the slaughterhouse level whether two conditions are respected. First, amplification of the risk has been previously demonstrated during the transport and lairage before slaughtering (5). So the bacteriological assessment is valid (as is the serological evaluation) only if the cross contamination is controled (by frequent and efficient cleaning and disinfection of truck and lairage). Second, environmental evaluation of a batch contamination involves that cleaning and disinfection are realized before the entry of the batch in the finishing room and the all in all out production systems must be achieved.

In the future, the assessment of the status of a batch will be a synthesis of serological and bacteriological data presented as a *Salmonella* score. The elaboration of this score is in progress. This score will be an efficient tool to evaluate the risk of a batch already at the farm level and to understand why and how contamination and persistence of *Salmonella* occur in a farm by an analytical epidemiological approach.

References

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Table1 : Mean results of six batches

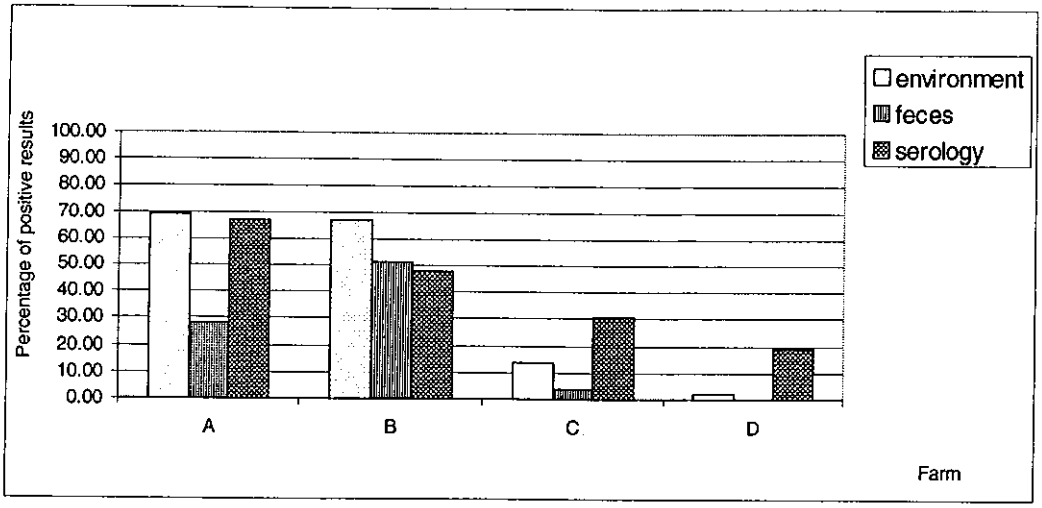

Table 2 : Data from successive batches from the farm A.

Date	10-jun	01-jul	16-sep	03-nov	28-nov	15-dec	TOTAL
Environment	3/7	6/7	0/7	7/7	7/7	6/7	29/42
%	**43**	**86**	**0**	**100**	**100**	**86**	**69**
Feces	3/10	4/17	1/10	5/17	6/17	6/17	25/88
%	**30**	**24**	**10**	**29**	**35**	**35**	**28**
Serology	7/14	8/16	7/14	9/16	16/17	16/17	63/94
%	**50**	**50**	**50**	**53**	**94**	**94**	**67**
Serotype	Derby	Derby	Derby	Derby	Derby	Derby	

Table 3 : Data from successive batches from the farm B.

Date	02-mai	02-jun	10-sep	13-oct	07-nov	08-dec	TOTAL
Environment	5/7	6/7	5/7	3/7	6/7	3/7	28/42
%	71	86	71	43	86	43	67
Feces	6/10	1/10	6/10	13/17	8/17	7/17	41/81
%	60	10	60	76	47	41	51
Serology	7/14	12/15	5/15	7/14	2/16	11/17	44/91
%	50	80	33	37	8	65	48
Serotype	Anatum	Anatum	Anatum	Anatum	Anatum	Anatum	

Table 4 : Data from successive batches from the farm C.

Date	24-apr	21-mai	18-jun	28-jul	24-sep	18-nov	TOTAL
Environment	0/7	0/7	0/7	3/7	2/7	1/7	6/42
%	**0**	**0**	**0**	**43**	**29**	**14**	**14**
Feces	0/9	0/11	0/17	1/10	1/17	1/17	3/81
%	**0**	**0**	**0**	**10**	**6**	**6**	**4**
Serology	2/15	0/13	2/15	12/15	10/15	1/15	27/88
%	**13**	**0**	**13**	**80**	**67**	**7**	**31**
Serotype	0	0	0	Typhimurium	Typhimurium	Typhimurium	

Table 5 : Data from successive batches from the farm D.

Date	12-nov	26-nov	13-jan	27-feb	06-apr	12-mai	TOTAL
Environment	0/7	0/7	0/7	0/7	1/7	0/7	1/42
%	**0**	**0**	**0**	**0**	**14**	**0**	**2**
Feces	0/17	0/17	0/17	0/17	0/17	0/17	0/102
%	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Serology	0/16	1/17	3/17	1/24	12/17		17/91
%	**0**	**6**	**18**	**4**	**71**		**19**
Serotype	0	0	0	0	Infantis	0	